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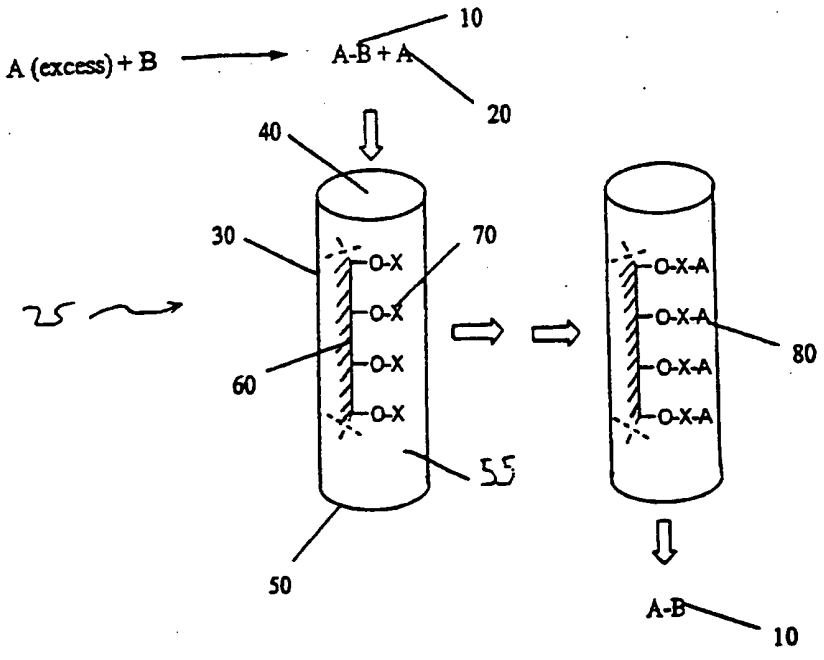
(54) Title: PURIFICATION DEVICE AND PURIFICATION METHOD



(57) Abstract: A purification device and purification method is described. The device (25) includes a quenching reagent (70) coupled to an insoluble support (60). A target compound (10) mixture flows across the quenching reagent (70), immobilizing impurity residues (20) on the quenching reagent (70) and purifying the target compound (10).

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PURIFICATION DEVICE AND PURIFICATION METHOD

This application claims priority from provisional patent application 60/153,630 filed September 13, 1999, which is hereby incorporated by reference.

Background of the Invention

The invention relates to a purification device for use in chemical synthesis and method of using the device.

Combinatorial chemistry and synthesis of chemical libraries have stimulated the development of synthetic strategies to rapidly and efficiently purify, isolate, and manipulate compounds during each synthetic step of forming the library members. For example, peptide, DNA, and small organic molecules libraries can be constructed using either solid phase or solution phase synthetic methodologies. In principal, solution phase synthesis could produce higher purity and lower cost final product than solid phase synthesis. In practice, solution phase synthesis can lead to the formation of an emulsion which can lower the purity and yield of the final product and variability of results.

Summary of the Invention

In one aspect, the invention features a method of removing a residue from a target compound mixture. The method includes contacting a target compound mixture including a target compound and a residue with a quenching reagent, flowing the target compound mixture across the quenching reagent, immobilizing the residue on the quenching reagent by forming a covalent bond between the residue and the quenching reagent, and removing the target compound from the quenching reagent. The quenching reagent is coupled to an insoluble support.

In another aspect, the invention features a method of deprotecting a target compound having a protecting group residue. The protecting group residue is an acid labile group or a base labile group. The method includes the steps of flowing the target compound mixture across a quenching reagent coupled to an insoluble support to allow the covalent binding of the protecting group residue to the quenching reagent, and removing the target compound from the quenching reagent. The protecting group residue can be a fluorenyl derivative.

In preferred embodiments the quencing reagent includes a piperazino, a piperidino, or functionally equivalent moiety.

In a preferred embodiment the quenching reagent can be coupled to the soluble support by a linker, e.g., a linear or aromatic linkers, e.g., a linker described

herein.

In a preferred embodiment the insoluble support can be an organic polymer or an inorganic oxide such as silica.

The method can include the steps of introducing the target compound mixture into a housing including the quenching reagent, and removing the target compound from the housing. Flowing can include moving a solvent through the housing or cartridge.

In another aspect, the invention features a device for purifying a target compound. The device includes a first opening and a second opening. The first opening and the second opening are connected by a fluid flow path. The device also includes a quenching reagent immobilized on an insoluble support contained within the fluid flow path. The quenching reagent is capable of forming a covalent bond with a residue. The residue can be a protecting group residue and the protecting group residue can be an acid labile group or a base labile group. The device can also include a second quenching reagent immobilized on a second insoluble support.

The residue can be a protecting group or an excess reagent. The residue and the target compound can be covalently bonded, such as when the residue is a protecting group. When the residue is a protecting group, the method can also include the step of releasing the residue from the target compound.

The insoluble support can be an organic polymer or an inorganic oxide such as silica.

The housing can include a cartridge. In addition to the insoluble support, the cartridge can include a solid phase support.

The quenching reagent can include a nucleophilic or an electrophilic group or a base, such as an amine, an isocyanate, an aldehyde, or an acyl halide. The quenching reagent can include a polystyrene functionalized with the quenching reagent or a silane functionalized with the quenching reagent.

The target compound can be a polypeptide, a nucleotide, or an organic compound. A polypetide can include, for example, 2 or more natural or unnatural amino acids. Nucleotides include, but are not limited to, any sugar-phosphate-based moiety, as well as any derivatized sugar-phosphate-based moiety. Examples of organic compounds, include but are not limited to, organic molecules containing a functional group protected by an acid or base labile group which reacts with the quenching agent to form a covalent bond.

The polymer-supported quenching reagents are functionalized polymer resins. The functional groups on the resins selectively bind to the residue via a covalent bond linkage.

Solution phase synthesis can offer certain advantages over solid phase synthesis. For example, a vast number of solution phase reactions have been developed in the synthesis literature, whereas relatively fewer reactions have been optimized for solid phase synthesis. The variety of protecting groups that can be used in solution phase synthesis overwhelms the relatively limited number of solid-phase synthesis resins. Solution phase synthesis can also lead to the elimination of resin attachment and cleavage steps during synthesis. In addition, solution phase synthesis is amenable to monitoring by ordinary chromatographic or spectroscopic techniques. Solid phase reactions can be more challenging to monitor.

Polymer-supported quenching reagents have improved the purity and reproducability of solution phase synthesis. For example, polymer supported reagents can be used as a rapid purification technique in solution-phase parallel synthesis. Depending on functional groups the desired products, by-products or solution-phase reagents can be selectively trapped on a resin. Previously used purification methods, such as crystallization, extraction, and chromatography can be difficult to apply when synthesizing a diverse library of compounds. Moveover, separating a structurally diverse array of compounds based on physical properties such as solubility or partition coefficient can present a formidable challenge when preparing libraries of compounds. The use of polymer-supported quenching reagents as a purification platform allows rapid solution phase synthesis to be carried out by eliminating the formation of emulsion by avoiding the need to perform liquid-liquid extraction steps.

Flowing a reaction mixture across a supported quenching reagent can overcome some of the difficulties encountered in solution phase method of treating a reaction mixture with a functionalized resin in a batch context. For example, time usage can be more efficient. The general procedure for removing the protecting group and purifying the solution phase product with polymer supported quenching reagents can requires between 14 hours and 4 days in a batch process. This reaction duration is long for combinatorial synthesis and purification situations. The reaction speed is significantly increased by flowing the target compound mixture across the quenching reagent coupled to an insoluble support. This exposes the reaction mixture to a higher surface area of the resin. The mixture experiences a greater amount of quenching reagent of the surface, increasing the overall rate of the reaction.

In addition, a batch process can require the use of a large excess of polymer resin. For example, typical solution phase methods require 1 mole of polymer resin per 0.2 mole of blocked product, significantly increasing the cost of the deblocking (e.g., deprotecting) step and the purification of the final product. By flowing the target compound mixture across the quenching reagent coupled to an insoluble support, less

resin can be used to achieve the same or improved purity of product.

Furthermore, batch processing can result in lower reaction yields due to transfer losses and material losses during filtration of the resin. Multiple transfers of the product and filtration result in loss of product. This can be particularly problematic if the loss occurs near the end of a multiple step synthesis. Flowing the target compound mixture across the quenching reagent coupled to the insoluble support increases the efficiency of the reaction and the purity of the product, thereby increasing reaction yields and purity.

Other features or advantages of the present invention will be apparent from the following detailed description and also from the claims.

Brief Description of the Drawing

FIG. 1 is a schematic diagram depicting a device and method for purifying a target compound mixture having an excess of a reagent.

FIG. 2 is a schematic diagram depicting a device and method for purifying a compound.

FIG. 3 is a schematic diagram depicting a device and method for purifying a target compound having a protecting group.

Fig. 4 is structural representation of several of reagents coupled to an insoluble support.

Fig. 5 is a diagram of reactions involved in Bsmoc removal.

Detailed Description

In general, the purification device includes a quenching reagent coupled to an insoluble support. For example, the quenching reagent can be covalently bonded to a polymeric support material. Alternatively, the quenching agent can be covalently bonded to an inorganic support material such as silica.

A target compound, which is a product of a synthetic reaction, can be separated from unwanted residues in a target compound mixture by selectively forming a covalent bond between the residue and the quenching reagent. For example, the residue can be excess starting material or a protecting group that has been removed from the target compound. The residue reacts with the quenching reagent to form the bond. This reaction immobilizes the residue on the insoluble support. Removal of the residue in this manner purifies the target compound once the target compound is removed from the quenching reagent coupled to the insoluble support.

By flowing the target compound mixture across the quenching reagent, the

target compound mixture is exposed to a continual supply of unreacted quenching reagent. The effective increase concentration of the unreacted quenching reagent increases the kinetics of the reaction between the residue and the quenching reagent. In general, the contact time between the target compound mixture and quenching reagent is increased. This arrangement can allow the reaction to proceed more rapidly and more completely. Coupling of the quenching reagent to the insoluble support permits the supported quenching reagent to be contained within a device, such as a disposable column. The device allows the target compound mixture to be passed through the column. Because the quenching reagent is coupled to the insoluble support which in turn is contained within the device, the target compound mixture can flow across the quenching reagent. Accordingly, the purification of the target compound can proceed in higher yield in a shorter amount of time.

The quenching reagent can be a functionality that can react to form a covalent bond with an electrophile (e.g., an amine which can react with an acyl halide or other electrophilic residue). Alternatively, can be a functionality that can react to form a covalent bond with an nucleophile (e.g., an isocyante which can react with an amine or other nucleophilic residue). The reagent-coupled insoluble support can be mixed with other solid support material. The other solid support material can be ordinary chromatography column packing material, such as organic polymer supports or inorganic oxide supports. Suitable support materials can include cross-linked polystyrene beads, silica, or reverse-phase silica.

The quenching reagent can be coupled by reaction with the surface functionality of the insoluble support. A commercially available an amine quenching reagent-coupled resin is Amberlyst 15 (Aldrich Chemical Co.). Quenching reagents (e.g., amines and isocyanates) can be coupled to a polymeric support as described, for example, in Booth and Hodges, *J. Amer. Chem. Soc.* 119:4882-4886 (1997) or in Knapczyk *et al. J. Org. Chem.* 48:661-665 (1983), each of which is incorporated herein by reference. Quenching reagents can be coupled to inorganic supports, such as silica, as described in Carpino *et al. J. Org. Chem.* 48:666-669 (1983), each of which is incorporated herein by reference.

The synthesis of a target compound can be facilitated by using reagents coupled to an insoluble support. When the reagent is a quenching reagent that can, for example, form a covalent bond with a protecting group of the target compound, the protecting group can be immobilized on the insoluble support. The reaction can be facilitated by exposing the compound to an excess amount of the coupled reagent. One way to facilitate the reaction is to flow a solution of the compound over the support containing the immobilized reagent. In particular, a reagent coupled to an insoluble

support can be packed in a disposable cartridge and the target compound can be exposed to the reagent by flowing a solution of the compound through the cartridge. The reaction can purify the target compound by removing the protecting group residue from the solution.

The insoluble support can be a resin or other chromatographic material, such as silica. The solution containing the target compound is a liquid phase. The insoluble support is a solid or stationary phase. As the solution containing the target compound and the protecting group is exposed to the immobilized reagent, adsorption occurs. Adsorption is the equilibrium distribution of target compound or the protecting group between stationary phase and the liquid phase. The adsorption capacity of the insoluble support or resin can be an important parameter to control to achieve adequate purification.

An excess of a starting material can be used in a synthetic reaction to drive the reaction to completion without fear of complicating isolation and purification of the final products by using a column including a coupled quenching reagent. Referring to FIGS. 1 and 2, an excess of reagent A (e.g., an isocyanate compound or acyl halide) is combined with reagent B (e.g., an amine compound) to form a target compound (e.g., an imide). The excess reagent is effectively removed by flowing the target compound mixture, which includes target compound 10 and excess residue 20, through device 25. Device 25 can be a cartridge or column used in chromatography.

Device 25 includes housing 30. Housing 30 has first opening 40 and second opening 50, connected by fluid flow path 55. Fluid flow path 55, within housing 30, contains insoluble support 60. Insoluble support 60 is coupled to quenching reagent 70 (e.g., an amine). Quenching reagent 70 is capable of forming a covalent bond with excess residue 20. Alternatively, the quenching reagent can be an electrophile (e.g., an isocyanate) which can form a covalent bond with an unreacted amine.

As the target compound mixture passes through fluid flow path 55, residue 20 passes across quenching reagent 70. Residue 20 reacts with quenching reagent 70, immobilizing residue 20 on the quenching reagent by forming a covalent bond 80. As a result, target compound 10 is obtained in high yield in a pure form. By flowing the target compound mixture through device 25, the quenching reaction occurs more rapidly than in a batch process.

Referring to FIG. 3, a target compound 10 bearing protecting group residue 20 can also be purified by the method. Protecting group residue 20 is base labile (i.e., is removed under basic conditions) and reacts to form a covalent bond with an amine. The protected compound can be purified by passing the target compound mixture, which includes protecting group residue 20 covalently bonded to target compound 10,

though device 25, including housing 30, and first opening 40 and second opening 50, connected by fluid flow path 55. As described above, fluid flow path 55, within housing 30, contains insoluble support 60. Insoluble support 60 is coupled to quenching reagent 70 (e.g., an amine). Device 25 can also include solid phase support 65 (e.g., other chromatography beads). Quenching reagent 70 is capable of forming covalent bond 80 with protecting group residue 20.

For example, a fluorenylmethoxycarbonyl (Fmoc)-protected amino acid was deprotected and purified using the device including a piperazine coupled to a silica support prepared as described Carpino *et al. J. Org. Chem.* 48:666-669 (1983). 200 mg of Fmoc-Ile-DCPM in 1 mL of DMF was injected into a Flash 12 cartridge (Biotage) packed with the silica-supported piperazine. The cartridge was washed with 10 mL of DMF at a flow rate of 0.4 mL per minute for an elapsed wash time of 250 minutes. HPLC analysis of the eluent showed nearly complete deprotection and no detectable dibenzofulvene residue. By comparison, 15% dibenzofulvene remains in solution when the protected reagent is stirred with the bulk reagent for five days. The reaction is more complete and proceeds more rapidly using the cartridge method.

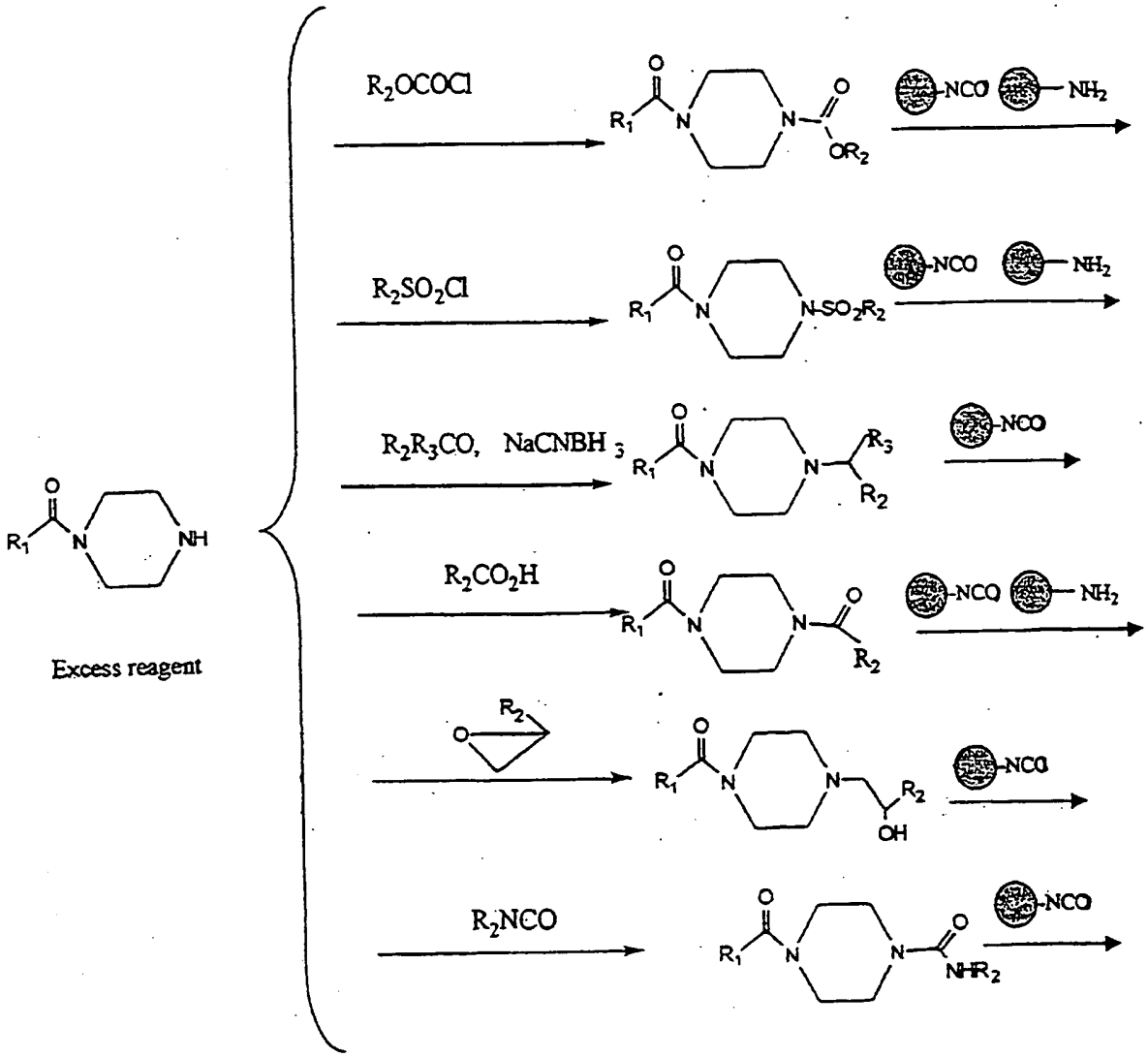
In another example, an alkylating reagent (e.g., an epoxide) can be used to synthesize a tertiary amine by alkylating a secondary amine. If the secondary amine is used in excess amount, an electrophilic quenching reagent, such as an isocyanate, which is coupled to an insoluble support in the device, can be used to purify the product by immobilizing the excess secondary amine on the quenching reagent.

In order to purify a target compound in a target compound mixture that can include a nucleophilic residue and an electrophilic residue (i.e., that have not reacted with each other), an electrophilic scavenger device and a nucleophilic scavenger device can be used sequentially. Alternatively, a mixed bed device can be used. In a mixed bed device, two or more layers of quenching reagent functionalized insoluble support can be used. For example, the device can have two layers. One layer can include an insoluble support having a quenching reagent capable of forming a covalent bond with a nucleophile. A second layer can be placed above or below the first layer. The second layer can include a coupled quenching reagent that is capable of forming a covalent bond with an electrophile. A solid phase support layer that is not functionalized can be placed in between the first layer and the second layer to prevent adverse interactions between the layers. The order of exposure each layer to the target compound mixture can depend on the nature of the residues in the target compound mixture. The order of exposure that provides the target compound in highest purity can be more efficient to use in the purification method, although this must be determined for each type of target compound mixture produced.

Scheme I depicts a series of synthetic reactions that can be carried out under excess reagent conditions to provide a target compound mixture that can be purified by the method using an electrophilic quenching reagent device, a nucleophilic quenching reagent device, or a mixed layer device.

5

Scheme I


Excess reagent
R2OCOCl
R2SO2Cl
R2R3CO, NaCNBH3
R2CO2H
R2NCO
NCO
NH2
OH

Example

Four non-swelling silica-base reagents were prepared and compared for carrying out de-blocking/scavenging reactions by continuous methods, e.g., column, and batch technique. Silica reagents bearing the piperazino and piperidino function were prepared. These organic bases were attached to silica using linear and aromatic linkers. Fig. 4 shows the four linker/reagent combinations.

Piperazino and piperidino functionalized silica gel were synthesized and evaluated in batch and on-column procedures. For on-column use, disposable columns (Biotage Flash Samplets) were prepared. These products were designed to: (1) de-block the amino protecting group 9-fluorenylmethyloxycarbonyl (Fmoc); and (2) scavenge the dibenzofulyene liberated in the de-blocking process. The application of these functionalized silica reagents was also shown for 1,1-Dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl (Bsmoc) amine protecting group.

A comparison of column-based use with pre-packed samplets and batch technique is shown in this work. The mechanical simplicity and efficiency of the column-based approach make possible the rapid, parallel synthesis and purification of solution-phase syntheses. These cartridges offer an alternative to solid-phase organic synthesis in the practice of combinatorial chemistry.

De-blocking studies were performed with 9-fluorenylmethyl *p*-chlorocarbanilate (Fmoc-PCA) as a test probe. Chromatography conditions were as follows: column, C4 Vydac, 4.6X100mn; Eluant, A, H_2O+ CAN (95+5), B, ACN + H_2O (95+5); Gradient, B(30-100%) in 15 minutes; Detection, UV @ 240 nm; Flow, 2 ml/minutes.

1g of piperazino-benzyl-functionalized silica (Reagent 2) was packed into Biotage samplets. 30 mg, 60 mg, or 90 mg of Fmoc-PCA was dissolved in 2 ml of DMSO and deposited on samplet. The solution was left in the samplet for 30 minutes. The amount of de-blocking was dependent of sample load. The 30 mg and 60 mg trial gave 100% de-blocking 100%. At a 90 mg load 97% de-blocking was achieved. Scavenging was dependent on, time, sample load, and solvent composition. The 30 mg, 60 mg, and 90 mg trials gave, respectively, 90% 76% and 60% scavenging.

Batch and column technique were compared for de-blocking. In a batch trial, 5 g of reagent 2 + 5ml of DMSO + 300 mg of Fmoc-PCA were stirred at room temperature for 30 minutes. In a column samplet/on-column trial, 5 g of reagent was packed in a

samplet and 300 mg of Fmoc-PCA was dissolved in 5mL of DMSO and added to samplet. After 30 minutes the samplet was washed with 10 ml of ACN and collected mixture tested by HPLC. Higher de-blocking and scavenging was achieved using column-based Samplet technology. The batch process gave 66% scavenging and 98% de-blocking. The samplet/On-column gave 87% scavenging and 100% de-blocking. The samplet method minimized amount of solvent used to recover.

1, 1-Dioxobenzo[b]thiophene-2-ylmethyloxcarbonyl (Bsmoc) is a common base sensitive amino group protecting group. See Fig. 5. Bsmoc undergoes Michael-like addition by base to generate the free amine and the reactive intermediate (2). The intermediate (2) decays over 8-10 minutes to give the stable de-blocking product (3). Bsmoc undergoes simultaneous de-blocking and scavenging steps (scheme 2) Scavenging adduct (2) doesn=t leave the column. Only two equivalents of base are needed for complete liberation of free amine (product).

Bsmoc- *p*-chlorocarbanilate (Bsmoc-PCA) was used as test probe. 1g of piprazinobenzyl-functionalized silica (reagent 2) was packed in the samplet. 30mg, 60mg, 100 mg, or 130 mg of Bsmoc-PCA dissolved in 2 ml of DMSO placed on samplet. The solution was left in the samplet for 30 minutes. De-blocking was 100% complete for the 30 and 60 mg of samplet. 98% de-blocking was achieved with the 100 mg of sample. Bsmoc-de-blocking is dependent on, time, sample load, and solvent composition.

Piperazino-benzyl-functionalized silica (Reagent 2) was introduced for on-column de-blocking and scavenging of Fmoc and Bsmoc groups. This reagent is very successful in Biotage samplet format and allows the automation of parallel solution phase synthesis using Fmoc-chemistry. Base functionalized silica gel can be used in different applications including: synthesis of peptide using Fmoc- and Bsmoc- chemistry; solid phase organic synthesis; solid phase Knoevenagel catalyst. Unreacted excess starting material is selectively removed from reaction mixture.

Advantages of solid supported de-blocking reagents include: no loss of product due to formation of emulsion; eliminate extractions; decrease the quantity of solvents utilized; decrease the number of steps in the process including no filtration of solid support; and allow automation of parallel solution phase chemistry.

Other Embodiments

It is to be understood that while the invention has been described in

conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the claimed invention. For example, other reactive functionalities, such as activated esters, alkyl halides, alkyl triflates, malonate derivatives, dienes, or dienophiles, can react with a residue of the appropriate reactivity in a target compound mixture to form a covalent bond.

What is claimed is:

Claims

1. A method of removing a residue from a target compound mixture comprising:

contacting a target compound mixture including a target compound and a residue with a quenching reagent coupled to an insoluble support;

flowing the target compound mixture across the quenching reagent;

immobilizing the residue on the quenching reagent by forming a covalent bond between the residue and the quenching reagent; and

removing the target compound from the quenching reagent.

2. The method of claim 1, wherein the residue is a protecting group.

3. The method of claim 1, wherein the residue is an excess reagent.

4. The method of claim 1, wherein the residue and the target compound are covalently bonded.

5. The method of claim 1, further comprising introducing the target compound mixture into a housing including the quenching reagent; and removing the target compound from the housing.

6. The method of claim 5, wherein the housing includes a cartridge.

7. The method of claim 5, wherein flowing includes moving a solvent through the housing.

8. The method of claim 3, further comprising releasing the residue from the target compound.

9. The method of claim 1, wherein the insoluble support is an organic polymer.

10. The method of claim 1, wherein the insoluble support is an inorganic oxide.

11. The method of claim 5, wherein the cartridge further includes a solid phase support.

12. The method of claim 1, wherein the quenching reagent includes an amine.

13. The method of claim 1, wherein the quenching group includes an isocyanate.

14. The method of claim 1, wherein the quenching reagent includes a polystyrene functionalized with the quenching reagent.

15. The method of claim 1, wherein the quenching reagent includes a silane functionalized with the quenching reagent.

16. The method of claim 1, wherein the target compound is a polypeptide.

17. The method of claim 1, wherein the target compound includes a nucleotide.

18. A method of deprotecting a target compound having a protecting group residue, the protecting group residue being an acid labile group or a base labile group, the method comprising:

flowing the target compound mixture across a quenching reagent coupled to an insoluble support to allow the covalent binding of the protecting group residue to the quenching reagent; and

removing the target compound from the quenching reagent.

19. The method of claim 18, further comprising introducing the target compound mixture into a housing including the quenching reagent; and

removing the target compound from the housing.

20. The method of claim 19, wherein the housing includes a cartridge.

21. The method of claim 20, wherein flowing includes moving a solvent through the cartridge.

22. The method of claim 18, wherein the insoluble support is an organic polymer.

23. The method of claim 18, wherein the insoluble support is an inorganic oxide.

24. The method of claim 18, wherein the quenching reagent includes a base.

25. The method of claim 18, wherein the target compound is a polypeptide.

26. The method of claim 18, wherein the target compound includes a nucleotide.

27. The method of claim 18, wherein the protecting group residue is a fluorenyl derivative.

28. A device for purifying a target compound comprising:

a first opening and a second opening, the first opening and the second opening being connected by a fluid flow path; and

a quenching reagent immobilized on an insoluble support contained within the fluid flow path, the quenching reagent being capable of forming a covalent bond with a residue.

29. The device of claim 28, wherein the residue is a protecting group residue, the protecting group residue being an acid labile group or a base labile group.

30. The device of claim 28, wherein the insoluble support includes silica.

31. The device of claim 28, wherein the insoluble support includes an organic polymer.

32. The device of claim 28, wherein the immobilized quenching reagent includes a polystyrene functionalized with the quenching reagent.

33. The device of claim 28, wherein the immobilized quenching reagent includes a silane functionalized with the quenching reagent.

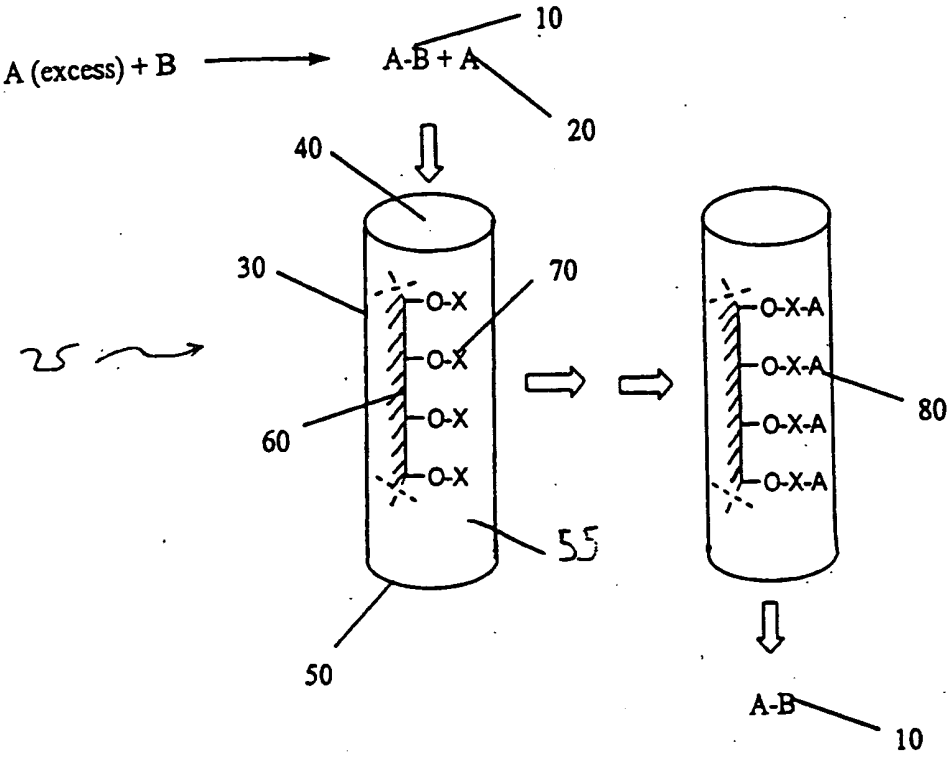
34. The device of claim 28, wherein the quenching reagent includes an amine.

35. The device of claim 28, wherein the quenching reagent includes an isocyanate.

36. The device of claim 28, wherein the device includes a housing.

37. The device of claim 36, wherein the housing includes a cartridge.

38. The device of claim 28, further comprising a second quenching reagent immobilized on a second insoluble support.


A (excess) + B
A-B + A
10
20
40
30
70
25
O-X
O-X
O-X
O-X
60
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50
O-X-A
O-X-A
O-X-A
O-X-A
80
A-B
10

FIG. 1

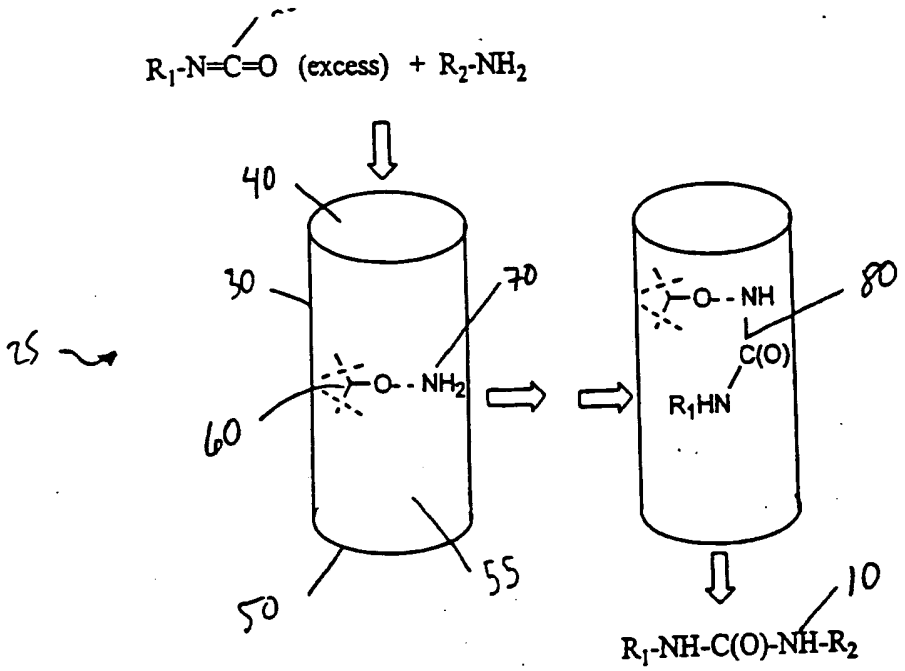
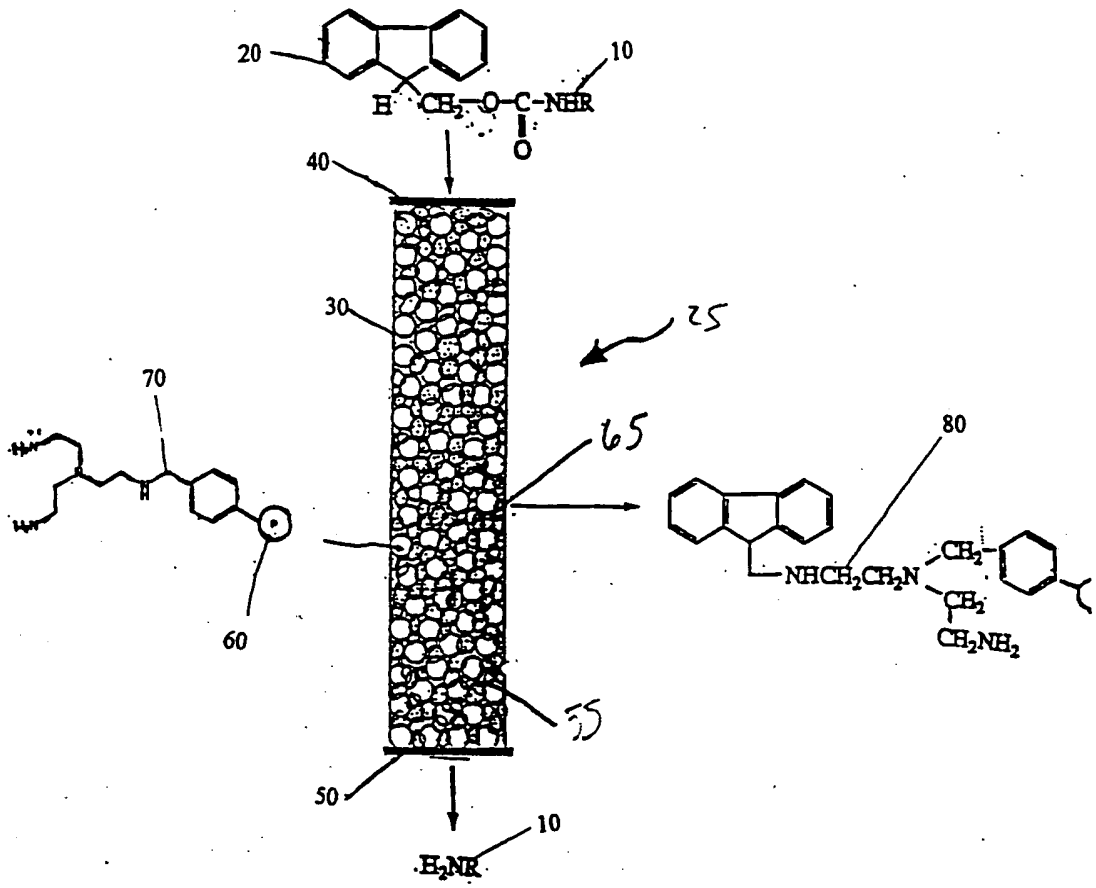

R1-N=C=O (excess) + R2-NH2
40
30
70
25
O - NH2
60
55
50
80
O - NH
C(O)
R1HN
10
R1-NH-C(O)-NH-R2

FIG. 2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NHR
O
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80
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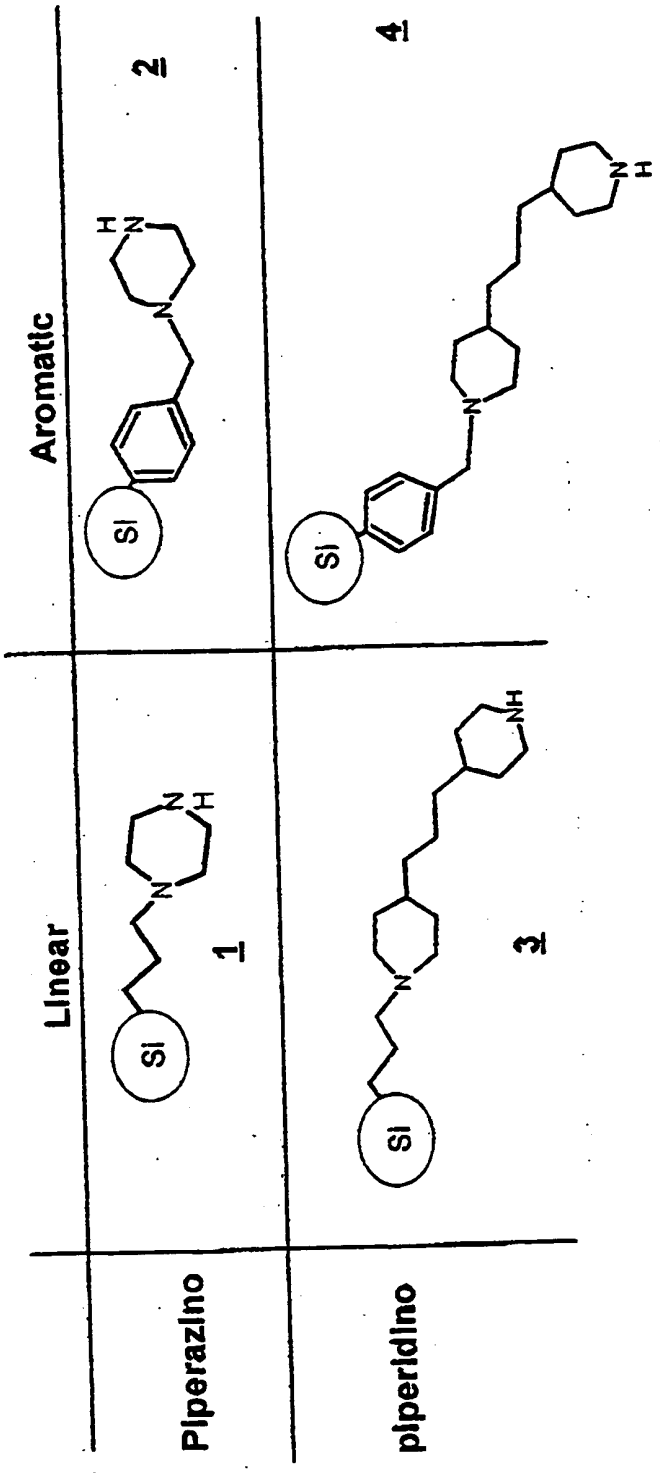
CH2
CH2
CH2NH2
55
50
10
H2NR

FIG. 3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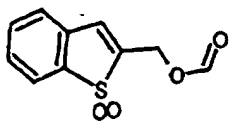
Linear
Piperazino
piperidino
1
2
3
4
Si
Si
Si
Si
N
H
N
H
N
H
N
H

Fig 4



Bsmoc-

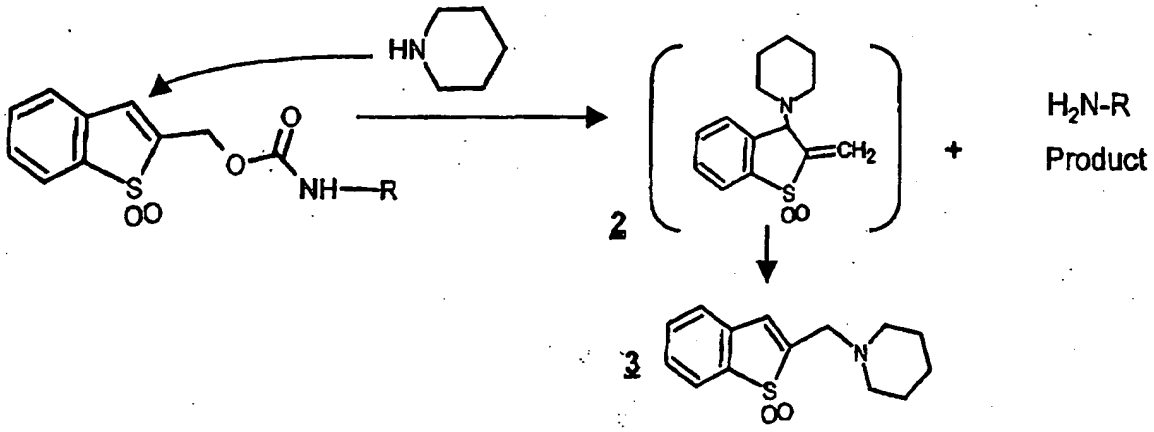


Fig 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/24978

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :B01D 15/08; C07K 1/16; C07H 1/06

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,965,289 A (SHERRINGTON et al.) 23 October 1990 (23.10.90), column 5, line 53 - column 6, line 27, column 7, lines 3-7.	1, 4-7, 9, 11, 28, 29, 31, 36-38
X	US 5,221,736 A (COOLIDGE et al.) 22 June 1993 (22.06.93), column 3, lines 55-66, column 4, lines 40-46, column 6, lines 25-42, column 16, line 15 - column 17, line 51, column 18, lines 28-50, column 19, lines 12-38.	28-31, 36-38
A	US 5,391,711 A (FUNAKOSHI et al.) 21 February 1995 (21.02.95), Figure 1.	1-38
X	US 5,405,938 A (SUMMERTON et al.) 11 April 1995 (11.04.95), column 64, lines 28-33.	28, 29, 31, 32, 34, 36-38

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 NOVEMBER 2000

Date of mailing of the international search report

29 DEC 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/24978

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,478,466 A (HEILMANN et al.) 26 December 1995 (26.12.95), claim 26.	1-38
X	US 5,576,453 A (BUESE) 19 November 1996 (19.11.96), column 1, lines 7-28, column 3, lines 14-27, column 4, lines 26-32 and 45-60.	28-30, 33, 34, 36, 37
A,E	US 6,121,488 A (NIKAM) 19 September 2000 (19/09/00), Abstract.	1-38
X	HARRIS et al. Protein purification methods: A practical approach. Oxford: IRL Press. 1989, pages 235-237.	28, 29, 31, 36, 37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/24978

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

210/263, 656, 665, 666, 668, 702, 710, 711, 749; 530/334, 335, 336, 337, 412, 415, 417; 536/25.31, 25.4

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

210/263, 656, 665, 666, 668, 702, 710, 711, 749; 252/182.34, 184; 521/53, 54; 525/342, 374, 379; 530/334, 335, 336, 337, 412, 415, 417, 810, 811, 812, 813, 814, 815, 816, 817; 536/25.31, 25.4

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, DIALOG
search terms: combinatorial library, deprotect, support, substrate, column, cartridge, isocyanate, amine, silane, polystyrene, quench, immobilize